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THE UTILIZATION OF FOODSTUFFS AND UREA PRODUCTION BY A LAND SNAIL DURING ESTIVATION

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Inhabitants of arid regions are periodically subjected to lengthy periods of drought. During times of low or even no rainfall considerable physiological demands are placed on a land snail. For instance, snails must have ample food reserves to assure survival because normal feeding may not resume for months. In addition the snails must endure high temperatures and conserve water. To escape desiccation terrestrial snails go into dormancy (estivation).

One aspect of the physiology of estivation that is of especial interest is the metabolic role of the accumulation of urea during dormancy (Horne, 1971). Why do some and not other snails buildup their urea levels? And what is the physiological importance of the urea when it is stored? Information on the metabolism of estivation in molluscs is generally scarce. Most of the studies have been conducted on the aquatic pulmonates (von Brand, 1931; von Brand, Nolan and Mann, 1948; von Brand, Baernstein, and Mehlman, 1950; von Brand, McMahon and Nolan, 1957; Coles, 1969) and on the prosobranchs (Meenakshi, 1958 and 1964).

To ascertain how one terrestrial pulmonate snail, *Bulimulus dealbatus,* has coped with the problems of water loss and starvation during estivation, the current study was initiated. The crux of the study was to answer the question of the importance of urea to the snail, and to determine if the urea production was related to the foodstuffs used during starvation or to osmotic stress.

Methods

Pulmonate snails, *Bulimulus dealbatus mooreanus* (Pfeiffer), were collected locally (Hays County, Texas) and were maintained in the laboratory at 22 ± 2.0° C and at a relative humidity ranging from 45–75%. Photoperiod was not controlled. Active snails were fed lettuce *ad libitum*. *Bulimulus* was identified by Dr. Joseph Rosewater, Curator, Division of Mollusks, United States National Museum.

Over an 80 day estivation period snails were weighed every 10 to 14 days. Periodically specimens were sacrificed and the wet weights and the dry weights determined to show the rate that tissue was metabolized. To get an accurate wet weight the whole snail was weighed; then by carefully removing and drying the shell to constant weight at 65° C, it was possible to obtain a good estimate of the wet weight. By drying the soft body at 65° C the dry weight was acquired.

In as much as the concentration of foodstuffs were changing in relation to each other, it was essential that the concentration be expressed in terms of an internal standard that was not affected by starvation (such as DNA). Thus an average DNA concentration was established for the active feeding snails. If, for instance, in an estivating snail the DNA increased by 25%, then a decrease in dry weight

of 25% would have had to have occurred to account for the higher DNA values. Therefore, it was possible to adjust the data on estivation to the initial concentrations of active feeding snails. This manipulation was indispensable to the expression of the foodstuff concentrations.

Total reducing polysaccharide in whole snails was prepared for analysis by the procedure presented by Oser (1965). The method of Nelson (1944) and Somogyi (1945) was used for color development and quantitative analysis. No attempt was made to distinguish between glycogen and galactogen.

Lipid in whole snails was homogenized in 10 ml methanol and then diluted with 20 ml chloroform (1 : 2) and separated according to the procedure of Sperry (1955). The lipid fraction was air dried in a weighing bottle and weighed to the nearest 0.05 mg.

TABLE I

Rate of weight loss of estivating Bulimulus dealbatus *in the laboratory**

Estivation in days	Wet wt. loss mg/g snail ± S.D. (N)	Dry wt. loss mg/g snail (10% of wet wt)	Dry wt. loss mg/g snail (calculated from DNA)
0	—	—	0.0 679 ± 103(5)**
17	99.7 ± 28.2 (12)	9.9***	11.0 789 ± 134(6)
26	127.7 ± 32.7 (12)	12.7	—
60	215.6 ± 39.1 (18)	21.5	20.1 880 ± 225(6)
80	312.0 ± 64.6 (11)	31.2	29.9 978± 218(5)

* Relative humidity = 45 to 78%; temperature = 22.0 ± 2.0° C.
** Actual DNA values [Mg DNA ± S.D. (number)].
*** Dry weight equals 10% of wet weight.

Total protein was precipitated with 10% trichloroacetic acid, centrifuged at 2000 × *g* for 10 minutes, the supernatant discarded and the precipitate redissolved in 1 N NaOH. For quantitative color development the method for Lowry, Rosebrough, Farr and Randall (1951) was used.

Lactic acid determinations were carried out by the Barker and Summerson method (1941) as presented in Oser (1965).

Urea was estimated by the colorimetric method of Archibald (1945) or the urease method of Conway (1957).

Measurement of aerobic respiratory rates was done with a conventional Warburg respirometer (Precision Scientific Co., Chicago) at 22.0 ± 1.0° C. It was unnecessary to shake the flasks since there was no fluid phase, except that of the 0.2 ml of 20% KOH. Thus, the snails were not distrubed by shaking (Cole,

1969). Active non-crawling snails were those individuals with the foot either extended or retracted, but without an epiphragm. Individual readings were made at 30 minute intervals and over a period of 2 to 3 hours.

Carbon dioxide and respiratory quotients were measured by the direct manometric method (Umbreit, Burris, and Schauffer, 1959).

Constant humidities were maintained with saturated salt solutions (Winston and Bates, 1960) in large vacuum desiccators. To assure adequate gas exchange with air the desiccators were left open. The published relative humidity values for the salt solutions employed here were checked with a Bacharach Instrument Co. (Pittsburg) humidity meter. The measured humidities were always within 1½% of the value reported by Winston and Bates (1960).

Results

During an estivation period of 80 days (relative himidity = 45–75%; temperature = 22.0 ± 2.0%) approximately 31% of the initial dry weight was consumed (Table I). Throughout estivation the ratio of dry weight to wet weight was approximately 1 : 10 (Table II), thus somewhat simplifying estimation of dry weight from the wet weight. Also by using DNA as an internal standard, it was possible to relate back to the initial conditions. For example, if the DNA content increased by one third on a gram basis, then a decrease in one third of the other tissue constituents would have had to occur. There was a little difference between the dry weight values calculated from percentage wet weight and DNA. The relatively consistent water content illustrated that evaporative water loss was replaced by metabolic water and that desiccation was not the most pressing physiological problem of estivating *Bulimulus*. In fact, the water content may have increased slightly. Active crawling snails had 87.0 ± 2.3% (15) water, whereas the estivating snails had about 89 to 91% water (Table II).

Table II

Influence of relative humidity on the water content and on the number of epiphragms formed by Bulimulus *after 58 days of estivation*

	KCl (85%)	$Ca(NO_3)_2$ (55%)	$MgCl_2$ (33%)	LiCl (14%)
Water				
$\bar{x}$ ± S.D.	91.2 ± 2.0	88.5 ± 2.6	89.3 ± 1.8	89.2 ± 2.7
	(6)	(6)	(6)	(6)
Epiphragms				
$\bar{x}$	2.0	3.5	2.7	3.7
(range)	(1–4)	(1–7)	(1–6)	(1–7)
(N)	(10)	(16)	(12)	(13)

A clear relationship between humidity and the number of epiphragms formed was not noted (Table II). Nevertheless, the snails in the highest humidity had fewer epiphragms than those in the lower humidities.

The rate of disappearance of foodstuffs during estivation is shown in Table III. Because carbohydrate, lipid and protein were changing in relation to one another

TABLE III

Utilization of foodstuffs during estivation

Foodstuffs (mg/mg DNA)	Feeding snail	Estivating snail (60 days)	Amount consumed in 60 days
Protein	86.1 ± 3.8 (20)*	67.8 ± 12.0 (21)	18.1 (12.3)**
Carbohydrate	13.2 ± 5.3 (10)	2.0 ± 1.6 (5)	11.2 (7.6)**
Lipid	13.3 ± 2.4 (12)	15.5 ± 4.1 (11)	—

* Mean ± standard deviation (number).
** MG consumed/g wet weight.

as they were utilized, their respective concentrations were expressed in terms of the internal standard DNA. By using DNA values to adjust the foodstuff concentrations of estivating snails to values comparable to those of active snails, it was possible to estimate the amount of foodstuff metabolized on a wet weight basis during dormancy.

Surprisingly little lipid was used, whereas carbohydrate and protein accounted for almost all of the dry weight loss. Protein made up 57% and carbohydrate 35% of the dry weight consumed for energy. Complete depletion of polysaccharide deposits (glycogen and galactogen) occurred in about 70 days (Fig. 1). It is not yet known if both lipid and protein or only protein are used once all of the

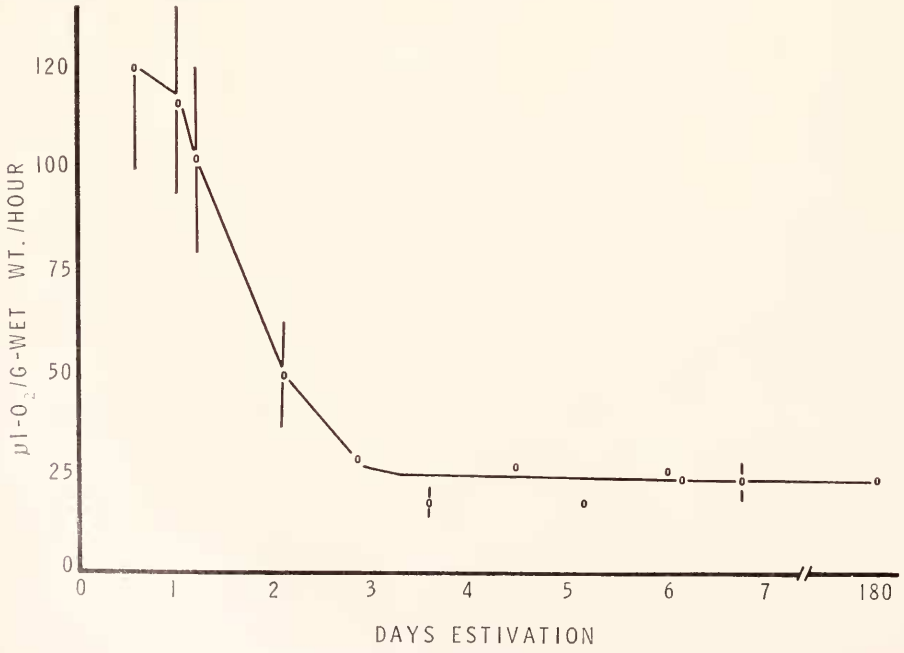

FIGURE 1. Rate of disappearance of reserve polysaccharide with estivation; Mean ± S.D. (Number ≥ 5)

carbohydrate reserve is gone. The average respiratory quotient for snails that have estivated for 120 days was 0.82.

The rate of oxygen consumption dropped sharply as the snails entered estivation, and more or less stabilized after three days at about 16% of the resting level (not crawling) (Fig. 2). The consumption of an average of 20 μl-0_2/g/hr by an estivating snail suggested that the snails were burning about 450 to 550 μg-foodstuff/g/day (one μl-0_2 = approximately one μg-protein; Cantarow and Schepartz, 1967). That some snails used as little as 2 μl-O_2/g/hr indicated that a few *Bulimulus* could withstand extended periods of dormancy. *Bulimulus* apparently did not seem to rely on anaerobic respiration since lactic acid values of only 170 ± 60 μg/g (10) were found in snails that had estivated for six months.

Upon being exposed to a relative humidity of 85%, snails that had been in estivation for six months would become active within one to three hours. Such a quick response to a moist situation assures that *Bulimulus* can capitalize on such environmentally favorable conditions. In Central Texas the snails are out feeding for short periods about twice a month in the spring and fall, but during the hot summer they may remain dormant for 3 to 4 months, depending on the pattern of rainfall. *Bulimulus* is also dormant during the cool winter months of December, January and February. Since the snails are active only during or shortly after a rain, they rarely have the opportunity to feed for more than 24 to 36 hours. During short periods of dormancy, the snails probably depend on carbohydrate for energy.

In the laboratory *Bulimulus* that have fed for several weeks store only 8.8 ± 3.5 mg-polysaccharide/g wet weight (10). The largest value recorded was 14 mg/g wet weight. It is doubtful that such deposits would be attained outside of the laboratory.

Humidity had an unexpected effect on the accumulation of urea during dormancy in as much as the response to humidity did not seem to be related to

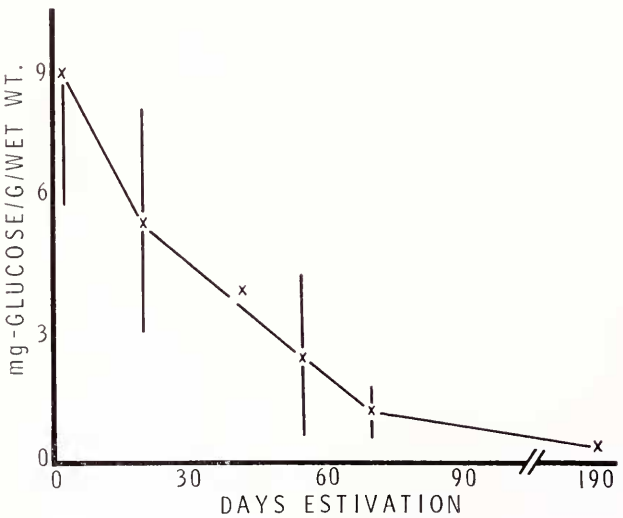

FIGURE 2. Changes in aerobic respiratory rates with estivation, Mean ± S.D. (N = 5)

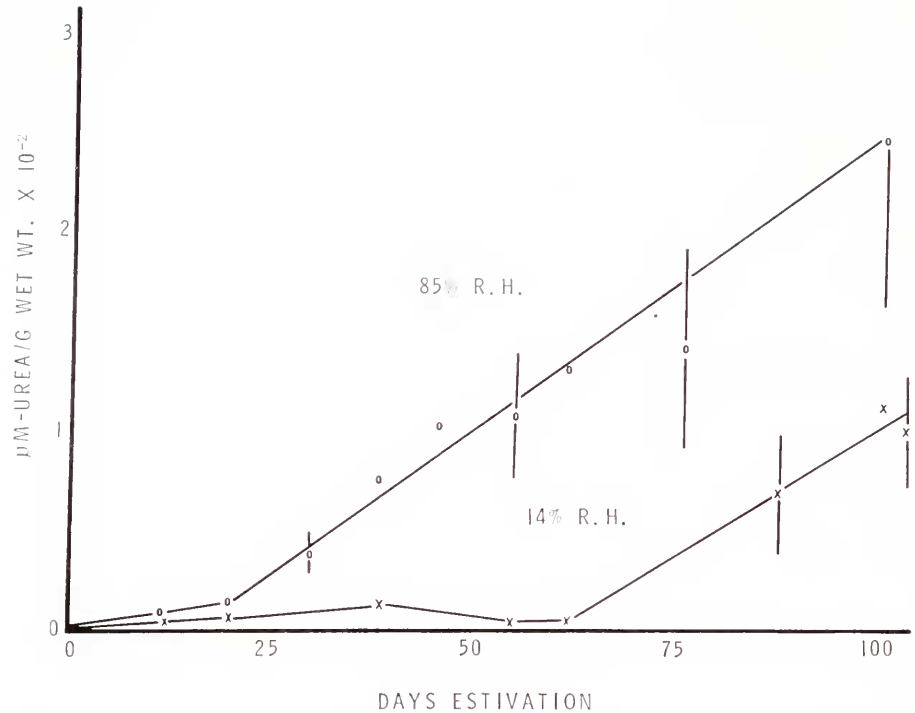

FIGURE 3. The effects of estivating at different relative humidities on urea accumulation. Mean ± S.D. (N = 5) The regression lines were calculated by least squares.

water loss, but rather to the activity of the animal (Fig. 3). In an atmosphere over saturated KCl (85% RH) *Bulimulus* remained active for two weeks before going into estivation. Yet even after two weeks, sixty-four of seventy-five snails were inactive and without epiphragms, while 11 had formed epiphragms. In an atmosphere over saturated LiCl (14% RH) all of the snails were estivating within nine hours. Since the buildup of tissue urea was greater at the higher humidities, the elevated urea values could not have been due to a rsponse to osmotic stress, but seemed only to further illustrate the metabolic demand on protein catabolism during starvation.

That relative humidity has little effect on dehydration of *Bilimulus* is illustrated in Table II. At all of the humidities studied the ratio of dry weight to wet weight remained constant. Hydration of the tissues was maintained by metabolic water.

DISCUSSION

Since protein declined the most during estivation and the estivating respiratory quotient was 0.82, the accumulation of urea with dormancy may be due in part to the enhanced catabolism of tissue protein. Apparently, some protein is used at about the same time that polysaccharide is mobilized. The carbohydrate reserves of snails fed *ad libitum* for at least 10 days, however, last only for about 70 days at

22° C. In Southwest Texas it would be unlikely that *Bulimulus* would ever have the opportunity to store so much carbohydrate.

During starvation and desiccation the aquatic pulmonate, *Australorbis*, depleted its polysaccharide and lipid stores, and seemed to depend on protein during extended periods of dormancy (von Brand, McMahon and Nolan, 1957). In their study the most pronounced reduction in carbohydrate and lipid occurred in the first ten days. On an annual basis the lipid content of *Helix pomatia* fluctuated only slightly while glycogen was stored prior to estivation and then consumed thereafter (von Brand, 1931). In other examples of the utilization of food stores, the aquatic snail, *Planorbis*, consumed mostly carbohydrate, while the desert pulmonate, *Sphincterochila*, appeared to use mainly carbohydrate and protein (Emerson,, 1967; Schmidt-Nielsen, Taylor and Shkolnik, 1971). At high humidities *Bulimulus* appeared to start utilizing a lot of protein by the fourteenth day, and therefore was somewhat like *Australorbis*.

That aerobic respiration persisted throughout estivation and that no lactic acid accumulated in the tissues was not especially surprising for a terrestrial snail. Both of the helicid snails, *Helix* and *Sphincterochila*, seemed to respire aerobically during estivation (Fischer, 1931; Schmidt-Nielsen, Taylor and Shkolnik, 1971). Even the aquatic pulmonates studied by von Brand *et al.* (1948) that have a limited capacity for anaerobiosis are also primarily aerobic.

The decrease in respiratory rate to about 16% of the normal resting rate demonstrated that the snails had entered a resting state. However, this state could be interrupted easily by placing the snails in an atmosphere with a relative humidity of 85%. At such a humidity all of the snails would be actively crawling within one to three hours. Both Fischer (1931) and Meyer and Thibaudet (1937) reported that *Helix pomatia* became inactive at any time of the year if food and water were removed, while activity resumed if food and water were provided. Following the onset of dormancy *Australorbis*, *Bulinus* and *Sphincterochila* reduced their respiratory rates between 9 and 30% of the initial rate (von Brand, McMahon and Nolan, 1957; Coles, 1969; Schmidt-Nielsen, Taylor and Shkolnik, 1971). *Australorbis* also showed a reduction in heart rate. A reduction in respiratory rate would be essential if such snails as *Bulimulus* are to survive many months in estivation. With an average oxygen consumption rate of 20 μl/g/hr, a one gram snail (130 mg dry weight) would use about 450–550 μg-tissue/d (one μl-O_2 = approximately one μg protein). At 22° C this means that *Bulimulus* would metabolize 50% of its dry weight within 4–6 months. Those individuals with respiratory rates lower than the average could undoubtedly estivate for several months longer. The desert snail, *Sphincterochila*, can estivate for longer periods than *Bulimulus* and seems to metabolize all tissue components (Schmidt-Nielsen, Taylor and Shkolnik, 1971) as does *Bulimulus*. At a relative humidity of 96% and at a temperature of 27° C *Australorbis* consumed 50–60% of its dry wt within 128 days (von Brand, McMahon and Nolan, 1957).

The catabolism of protein for energy is not surprising when the activity patterns of *Bulimulus* are considered. For example, *Bulimulus* is out actively feeding only during or shortly after a rain. At most, the snails feed for about 24 to 36 hours and are starving the rest of the time. In other words, *Bulimulus* spends most of its time in estivation in semi-arid Central Texas, U.S.A. With such short periods

of feeding it is doubtful that *Bulimulus* can ingest enough food to form large polysaccharide deposits. Even in the laboratory where lettuce is fed *ad libitum* the concentrations of carbohydrate was extremely variable. The highest concentration was 14 mg/g snail. Polysaccharide values in other species are generally much higher. Glycogen values in *Pila* ranged from 20.2–24.5 mg/g (Meenakshi, 1958), and for four aquatic pulmonates, von Brand, Baernstein and Mehlman (1950) reported values of 10 to 35 mg/g snail. These data show that *Bulimulus* deposits much less polysaccharide than many other snails. The high individual variation in reserve carbohydrate may explain the differences in the amounts of urea that accumulate during estivation. That is, snails with a high food reserve would catabolize less protein and thus need to detoxify less ammonia than a snail with a low polysaccharide reserve.

That low humidity does not enhance the production of urea clearly illustrates that osmotic stress does not effect the control of urea biosynthesis in snails as it may in some animals (McBean and Goldstein, 1971). In a relative humidity of 14%, where the threat of water loss by evaporation was greatest, the production of urea was least. The much more rapid buildup of urea in the environment of 85% R.H. was perhaps due to a more rapid mobilization and breakdown of protein. During estivation the end-product of nitrogen catabolism seems to be urea, not ammonia or uric acid (Horne, 1971). At the high humidity, starving *Bulimulus* remained actively crawling for about two weeks before estivating, while at the lower humidity the snails were all estivating within nine hours. Carbohydrate reserves would be metabolized much sooner in the active crawling snails.

High urea levels could conceivably be of some importance in reducing evaporative water loss in a snail buried in the soil. Urea concentrations of 300 μmoles/g would increase the osmotic pressure of a snail by about 6 atmospheres. However, since the water content of the snails that had estivated 58 days in humidities ranging from 14 to 85% did not differ, it is unlikely that the role of urea is related to water conservation. Apparently, the epiphragms in *Bulimulus* are what really reduceds water loss, even though the number of epiphragms formed did not seem to be affected by humidity. The numerous chambers formed by the epiphragms may reduce evaporation by decreasing air circulation in the spaces between epiphragm. Machin (1968, 1972) discussed the permeability of helicid epiphragms to water and has emphasized the importance of the mantle in reducing evaporative water loss, whereas the operculum of *Pila* was found by Meenakshi (1964) to be critical in decreasing water loss in this species.

Water loss is not the most critical problem encountered by estivating *Bulimulus*. But rather, it is the depletion of food reserves that determines how long a snail may remain dormant. The reduction in respiratory rate allows for prolonged periods of dormancy. The physiological implications of the elevated urea levels are uncertain, except that they seem to be related to protein catabolism, ammonia detoxification and the fact that the snails are not voiding urine during inactivity.

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Summary

1. During a sixty day estivation period, the land pulmonate, *Bulimulus dealbatus,* metabolized 21.5 mg-tissue/g dry wt. Of the tissue consumed, protein and polysaccharide made up 57% and 35% of the loss.

2. By the third day after onset of estivation aerobic respiratory rates declined to 16% of the resting level.

3. Snails maintained in a 85% R.H. were active longer and accumulated urea faster than those snails in 14% R.H. The high concentrations of urea were probably related to an enhanced catabolism of protein and not to osmotic stress.

4. The water content of *Bulimulus* and the number of epiphragms formed was not correlated with the estivation humidity.

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